technical briefs

Diurnal Variation of Urinary "hCG β Subunit Core Fragment" Production Evaluated in Patients with Gynecological Neoplasms, *Patrick Neven,[1,2] Ray K. Iles,[3] Paul G. Carter,[1] John H. Shepherd,[1] and Tim Chard[3]* [[1] Gynecol. Oncol. Unit, St. Bartholomew's Hosp. and the Royal Marsden Hosp., London; [2] Algemene Kliniek St. Jan, Broekstraat 1000, Brussels, Belgium (address for correspondence), fax 32-2-219-86-96; [3] Williamson Lab. for Molec. Oncol., St. Bartholomew's Hosp., London]

Urinary metabolites of the β subunit of human chorionic gonadotropin (hCGβ), particularly the fragment known as β-core, are potential tumor markers for gynecological cancers (*1–6*). We have found that an increased concentration (μg/L) of β-core in early-morning urine samples is relatively common in gynecological cancers, but the sensitivity (36%) and specificity (90%) of such measurements are low (*7*). Most clinical studies have reported β-core concentrations in early-morning or random urine specimens without correction for urine volume or concentration (*1–7*). For many urinary solutes, however, including proteins, expressing the concentration as a ratio to the urinary creatinine concentration is less variable and may better reflect the true urine output than does concentration per unit volume (*8–11*).

In the present study, we examined the variation of urinary β-core excretion over a 24-h period, to determine whether expressing β-core results as a ratio to creatinine or to total volume of urine gave results that were less variable than concentrations per unit volume for any given early-morning or random specimen.

Urine samples were obtained preoperatively from 35 women, ages 28–66 years. All were hospitalized for surgical treatment of a benign (n = 8) or malignant (n = 27) gynecological condition. The patients had serum creatinine concentrations within the normal reference range, were in stable condition, and were selected from an inpatient population on the basis of their willingness to cooperate with the study. The procedures followed were in accordance with the ethical standards of our institution's responsible committee. The women collected a portion from each urine specimen passed during 24 h; 20 of these women recorded both the volume and the time of each micturition. Sodium azide (1 g/L) was added to the unprocessed urine, which was stored at −20°C until analysis.

Results on 16 women were excluded: 11 because of failure to conform to the collection protocol and 5 because there was no measurable β core in an early-morning urine specimen. For the 24-h analysis, a complete set of samples was obtained in 19 women (136 specimens, an average of 7 per subject).

We measured β-core by RIA (detection limit 0.1 μg/L) as described previously (*12*). In this assay, β-core shows partial cross-reactivity with intact hCG (6.9%) and free hCGβ (18%) and negligible cross-reactivity for luteinizing, thyroid-stimulating, and follicle-stimulating hormones (<0.7%). Creatinine was measured by a kinetic Jaffé method with a Hitachi 717 analyzer. The specimens from each woman were thawed and analyzed in a single assay. One analyst performed all the assays, and the same batches of reagents were used throughout. The between-assay variability (CV) ranged from 2.3% to 13.4% for β-core (0.1 to 5 μg/L) and from 2.5% to 2.7% for urine creatinine (3.9 to 7.1 mmol/L). The within-assay variability ranged from 2% to 12.5% for β-core and from 1.1% to 1.5% for creatinine.

The concentration of β-core was expressed as either μg/L, mg/mol of creatinine, or μg/h. The CVs for the three indices were compared by using one-way analysis of variance.

Variations with time (CVs) in individual women ranged from 14.9% to 103% (mean 45.2%) for β-core concentration, from 7.4% to 43.6% (mean 19.1%) for β-core per mole of creatinine, and from 9.6% to 42.6% (mean 21.9%) for β-core per unit of time. The CV for concentration (μg/L) was significantly greater than the CVs for β-core expressed per unit of creatinine or time ($P < 0.01$). There was no evidence for any systematic early-morning peak or time-related pattern in any of the three indices, although six subjects had their highest β-core concentration (μg/L) in the early-morning specimen. Four women had no measurable β-core in the whole 24-h collection. Urine β-core and creatinine concentrations were closely related; for all patients, the coefficient of linear correlation (r) ranged from 0.732 to 0.997. In five cancer patients, from whom at least six measurements of urinary β-core exceeded the RIA detection limit, we found a modest to strong correlation with creatinine content (r = 0.666 to 0.985) and a significant fit to a linear regression model (P = 0.07 to <0.0001). However, when all data were combined, the correlation decreased (r = 0.402) because of the variable output of β-core from each individual patient. Hourly β-core excretion and β-core per unit creatinine in early-morning samples were also related (r = 0.756).

The advantages of reporting concentrations of substances excreted in urine after adjustment for urine concentration has been shown for several different solutes (*8–11*). Other urine tumor markers, e.g., tumor-associated antigen, show a systematic diurnal excretion pattern (*13*). In this study we were not able to identify a systematic diurnal rhythm in urine β-core production in women with gynecological neoplasia, similar to observations made during the first trimester of pregnancy (*14*).

Paterson (*15*) noted that creatinine output is not sufficiently constant to act as a reference against which the excretion of other solutes can be standardized, and suggested that the time-related excretion rate of a solute should be more reliable than this. By contrast, the present data show that time-to-time variation is equally reduced by expressing results per unit of creatinine or per unit of time. However, we recognize that adjustment for urinary creatinine within an individual does not allow for systematic differences in creatinine excretion between individuals, e.g., because of differences in lean body mass.

The clinical importance of the present findings is that measurement of β-core in a single early-morning or random urine specimen is likely to give a more accurate result when the value is related to creatinine concentration. Whether this index will better distinguish between the benign and malignant group and whether creatinine-corrected β-core is a better prognostic indicator are the subjects of on-going studies.

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Rapid Screening for *p53* Mutations with a Sensitive Heteroduplex Detection Technique,

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Genetic changes such as point mutations, rearrangements, and amplification or deletion in a single cell may result in malignant transformation. It is widely accepted that mutations in the *p53* tumor suppressor gene are among the most frequent alterations that occur during the malignant progression of many tumor types (*1, 2*). The product of *p53* is a 393-amino acid nuclear phosphoprotein that was first described in 1979. Most mutations in *p53* occur in exons 5 through 8 in four evolutionarily conserved domains (*1*). Mutations have been shown to cluster to these conserved regions of *p53* in many human tumors, including breast, colon, lung, brain, and leukemia/lymphoma.

Li-Fraumeni syndrome (LFS) is a rare condition in which affected family members develop many different types of tumors similar to those sporadic tumor types that contain *p53* mutations (*3*). Germline *p53* mutations were first described in LFS patients who developed breast carcinomas, sarcomas, and brain tumors.

We examined the ability of a heteroduplex technique to detect single-base mutations in cells from two previously characterized LFS patients.

Two human fibroblast cell lines, MDAH041 and MDAH087, derived from two LFS patients were obtained from Michael Tainsky (University of Texas–MD Anderson Cancer Center, Houston, TX) (*4*). MDAH041 contained a single-base deletion at codon 184 (exon 5), and MDAH087, a point mutation at codon 248 (exon 7). The cells were grown as previously described (*5*).

We resuspended a cell pellet containing 1×10^6–2×10^6 fibroblasts in 200 μL of extraction buffer (50 mmol/L Tris-HCl, pH 7.6; 100 mmol/L NaCl; 1 mmol/L EDTA; 5 g/L sodium dodecyl sulfate) and digested it overnight with 290 mg/L proteinase K at 37°C. The samples were then incubated for 10 min at 65°C to inactivate the proteinase before being exposed to RNAse A for 1 h at 37°C. DNA was ethanol-precipitated after the addition of saturated NaCl and resuspended in 10 mmol/L Tris–1 mmol/L EDTA, pH 7.6.

We incubated genomic DNA (0.5–1.0 μg), isolated as described above, in a total reaction volume of 100 μL containing 300 ng of both the forward and reverse exon-specific primers, 2.5 U of Taq polymerase, 200 nmol/L each deoxynucleotide triphosphate, 1.0 mmol/L $MgCl_2$, 67 mmol/L Tris-HCl (pH 8.8), 10 mmol/L 2-mercaptoethanol, 16.6 mmol/L ammonium sulfate, and 6.7 μmol/L EDTA. The primers used were as follows: exon 5, 5′GTTCACTTGTGCCCTGACTT3′ and 5′AGGAATCAGAGGCCTGGGGA3′; exon 7, 5′TGCTTGCCACAGGTCTCC3′ and 5′AACCACCCTTGTCCTTTCTG3′. DNA was initially denatured at 94°C for 6 min before amplification. Polymerase chain reaction (PCR) amplification was accomplished with 35 cycles consisting of 2 min annealing at 55°C, 3 min extension at 72°C, and 1 min denaturation at 94°C. The final cycle included a 2-min annealing step at 55°C and a 10-min extension step at 72°C.

The PCR-amplified product from each patient was heat-denatured at 100°C in a beaker of water for 3 min and then slowly cooled to 45°C by allowing the sample to remain in the water at room temperature on the benchtop. We mixed an aliquot of 40 μL of this product with 6 μL of gel loading buffer and electrophoresed the product on a 38-cm vertical Hydrolink-MDE gel (AT Biochem, Malvern, PA) that was 1.5 mm thick. We diluted the MDE gel to a 1× concentration (from 2× stock) in 0.6× Tris–boric acid–EDTA (TBE) buffer (1× = 133 mmol/L Tris, 81 mmol/L boric acid, and 3 mmol/L EDTA) and 150 g/L urea. After gel polymerization, electrophoresis was carried out for 16 h at 500 V. The gel